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Crystal Structures of Catalytic Complexes of the Oxidative DNA/RNA Repair Enzyme AlkB

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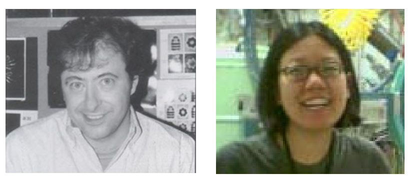
AlkB is a protein whose role in DNA repair has only recently been elucidated. Identified as a member of the Fe(II)-2-oxoglutarate-dependent digoxygease superfamily, AlkB directly converts alkylated DNA and RNA bases back into their original form. The preferred substrates of AlkB are based modified by Sn2-type alkylating agents, like busulfan, an anti-cancer drug widely used in the treatment of chronic myelogenous leukemia. Here we describe the crystal structure of substrate and product complexes of E. coli AlkB. Anaerobic crystallization conditions were used to obtain structures from a protein construct optimized for crystallization based on high-resolution backbone amide $^1H/^2H$ exchange measurements.

Enzymes in the Fe(II)-2-oxoglutarate-dependant digoxygenase superfamily use molecular oxygen, Fe(II), and the decarboxylation of 2-oxoglutarate (2OG) to generate succinate and CO_2 while oxidizing organic substrates. This superfamily makes up the largest class of non-heme iron-containing enzymes and is responsible for such diverse biological reactions as the synthesis of some antibiotics and plant metabolites, the hydroxylation of collagen, and the regulation of hypoxia. In the reaction catalyzed by AlkB, a methyl adduct on a nucleotide base is converted to an unstable hydroxymethyl moiety that is spontaneously released as formaldehyde to regenerate the original unmethylated base. Several AlkB substrates have been identified: 1-methyladenine, 3-methylcytosine, 1-methylguanine, and 3-methylthymine. *E. coli* AlkB has also been demonstrated to remove ethyl, propyl, and exocyclic etheno adducts. The substrates for AlkB are generated by S_N2 (substitution, nucleophilic, bimolecular) alkylating reagents preferentially in single-stranded DNA and RNA. However, AlkB has been shown



Authors (from left) John Hunt and Bomina Yu

to demethylate these bases in both single-stranded and double-stranded nucleic acid polymers as well as in DNA/RNA hybrids.

The accumulation of DNA lesions caused by alkyl modifications to nucleic acids is associated with the development of aging, cancer, and neurodegenerative diseases. However, these same DNA modifiers are widely used as chemotherapeutic drugs for the treatment of cancer. Consequently, a thorough knowledge of how DNA repair mechanisms work could benefit both cancer therapy and prevention. Understanding how AlkB repairs different nucleic acid bases requires the detailed characterization of how substrates are recognized and bound in the active site. However, initial attempts to crystallize full-length *E. coli* AlkB proved unsuccessful. High-resolution backbone amide $^1H/^2H$ (H/D) exchange measurements showed that the N-terminus of *E. coli* AlkB is conformationally flexible. When repeated in the presence of iron and substrates, other internal regions of the protein backbone became significantly more protected against H/D exchange, implying that the presence of the ligands stabilized the structure. A new construct lacking the first 11 amino acids (AlkB-ΔN11) was created. Crystallization trials were performed in the presence of Fe(II), 2OG, and a model methylated trimer [dT-(1-me-dA)-dT] under anaerobic conditions since the presence of oxygen would cause enzyme turnover. This new N-terminally truncated AlkB produced high-quality crystals under these conditions. Crystals obtained anaerobically were exposed to atmospheric oxygen over a number of days to capture the enzymatic reaction in progress.

The resulting crystal structures of *E. coli* AlkB offer insights into the DNA repair mechanism catalyzed not only by AlkB but by Fe-2OG dioxygenases in general. Although the Fe-2OG dioxygenase core of AlkB-ΔN11 matches that in other superfamily members, a unique, conformationally flexible subdomain holds a methylated trinucleotide substrate into the active site. This subdomain corresponds to that region protected by H/D exchange in the presence of ligands. The flexibility of this "lid" may enable docking of diverse alkylated nucleotide substrates into the active site. The observed open and closed states of a tunnel (**Figure 1**) in different crystal structures provide further evidence for a putative O_2 diffusion pathway that has been suggested for other superfamily members. Furthermore, we found that exposing crystals of the anaerobic Michaelis complex to air yields partial oxidation of 2OG to succinate (**Figure 2**), supporting the theory that oxygen binds to the iron ion which then moves or rotates to catalyze the dealkylation reaction.

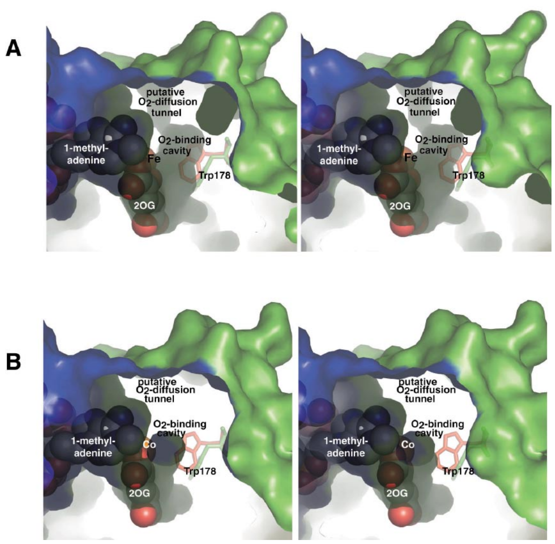


Figure 1. *Stereo pairs showing cavities near the active site in* **(a)** *the structure of the anaerobic Michaelis complex after 2 h of exposure to air and* **(b)** *the substrate complex with Fe(II) replaced by Co(II) and in which the putative O_2-diffusion tunnel is closed. The molecular surface of the protein is colored according to the subdomain of origin (with the nucleotide-recognition lid in blue and the catalytic core in green). The sidechain of trp-178 is shown in the different conformations observed in these two structures, which exhibit the greatest variation in the structure of the O_2-diffusion tunnel among the 7 crystal structures reported in our paper. Trp-178 has been reported to be hydroxylated by AlkB in the absence of nucleotide substrate, although no evidence of this modification is observed in the any of these refined crystal structures.*

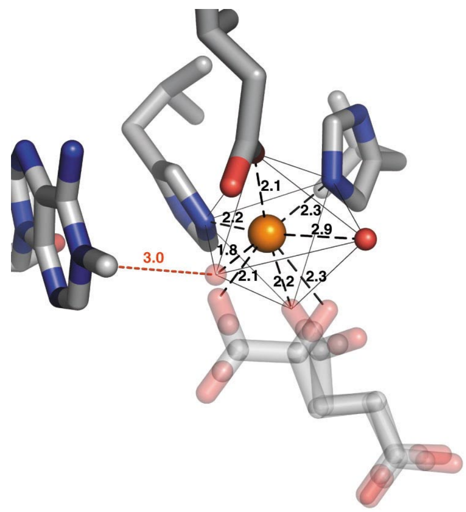


Figure 2. *Stereo pair showing active site stereochemistry in the crystal structure in which the anaerobic Micheaelis complex was exposed to oxygen for 2 h. Unbiased election density maps support the conclusion that a significant amount of the 2OG has been oxidized to succinate but that the adenine base remains largely methylated after short-term in situ oxidation. The alternative ligands are shown in semi-transparent rendering with the degree of transparency scaled according to refined occupancy.*